The Ortho Effect in Ligation of Iron Tetraphenylporphyrins

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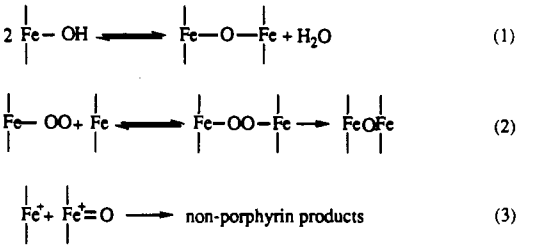
Kinetics and equilibria of binding of 1-methylimidazole, 1,2-dimethylimidazole, isonitriles, and carbon monoxide to iron(II) tetraphenylporphyrin and iron(II) tetramesitylporphyrin bearing various ligands in the sixth position have been determined. Those fifth and sixth ligand combinations which result in steric repulsion between the porphyrin and one of the ligands (e.g. 1,2-dimethylimidazole) show much greater binding constants and slower dissociation rates with the iron(II) tetramesitylporphyrin than to iron(II) tetraphenylporphyrin. Thus in the bis-(1,2-dimethylimidazole) complex the rate of dissociation from the iron(II) tetraphenylporphyrin system is about 10^4 times that for the iron(II) tetramesitylporphyrin. These large differences are attributed to a conformational effect in which phenyl groups with ortho substitutents destabilize the domed form of the metalloporphyrin. Possible consequences of this effect on catalytic and ligation behavior are discussed.

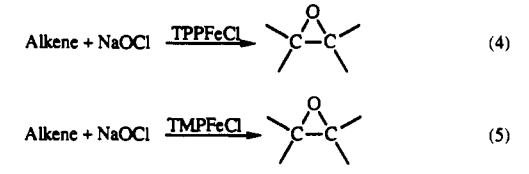
All natural porphyrins have the meso positions unsubstituted.[1,2] Therefore biomimetic studies of iron porphyrins have often employed this same class of porphyrins.[3-5] However, tetraphenyl porphyrins are easier to prepare and to elaborate than are the natural porphyrins.[6] Therefore an increasing number of arylporphyrins have recently been employed in biomimetic studies of dioxygen binding,[6-10] catalysis[11-16] and electron transfer reactions.[17-20] Furthermore, it is convenient to use ortho substituted phenyl groups to prevent formulation of the μ-oxo derivative[21] (eq 1), to prevent bimolecular oxidation of the Fe(II)[7] (eq 2), and to stabilize the iron(III)porphyrin toward oxidative destruction[11,13,16] (eq 3).



While these approaches are synthetically attractive and achieve the desired stabilities, they introduce structural changes from the natural porphyrins which might change interpretations of binding or catalytic functions. It is important to determine what, if any, effect the phenyl and ortho substituted phenyl groups have on the properties of the metalloporphyrins.

Chelated protoheme (**1**) binds carbon monoxide about five times and oxygen about 15 times more strongly than does chelated tetraphenylheme (**2**)[22] (Chart I). However, either picket fence heme–1-methylimidazole (**3**) or the corresponding chelated picket fence heme bind carbon monoxide about fifty times and oxygen over one hundred times more strongly than does chelated tetraphenylheme (**2**).[10,23] Tetramesityl heme, which has ortho methyl substitutents binds a second 1,2-dimethylimidazole several hundred times more strongly than does tetraphenylheme. This preference is also seen in the ferric porphyrins.[6,7]

In a kinetic study of epoxidations using tetraphenylhemin and tetramesityl hemin,[12-14,24,25]



the latter catalysts displayed larger rates and higher product selectivity and stereoselectivity.

All of these observations point to a special effect of ortho substituents in (iron) tetraphenylporphyrins which, in contrast

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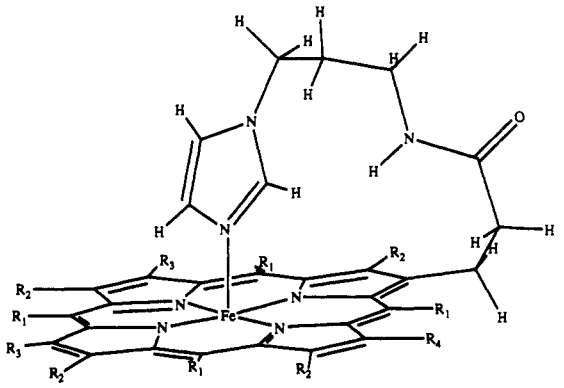
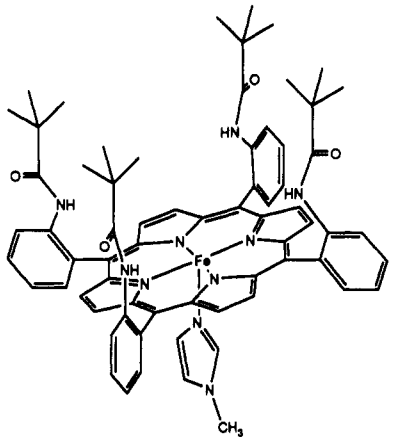
Chart I



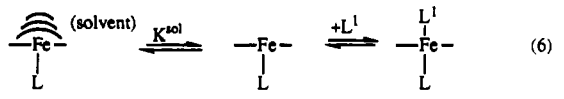
1 Chelated protoheme R_1=H, R_2=Me, R_3=vinyl R_4=$CH_2CH_2CO_2Me$
2 Chelated tetraphenyl heme R_1=Ph, R_2, R_3,R_4=H



3 α,α,α,α-tetra-o-pivalamidophenyl heme-1Methylimidazole complex

to expected steric effects, increase rates and equilibria for reaction at the metal. Two general proposals for this rather remarkable effect (which we term the "ortho effect") have been offered; a preferential solvent effect and a planarity effect.

In the solvent effect it is proposed that steric effects prevent solvation of the metal site, allowing free access to ligand.



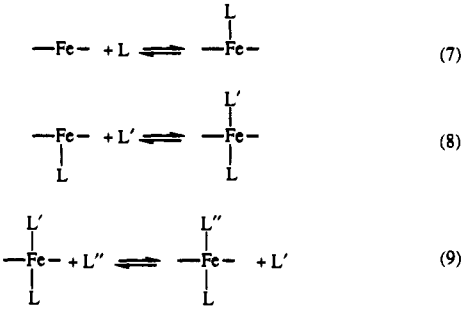
(6)

Thus ortho substituents reduce K^{sol} increasing the overall measured K^{L1} for ligand addition.

The planarity effect assumes that interactions of ortho groups with the heme plane both hinder the rotation of the phenyl group and reduce the doming of the porphyrin. Other effects such as hindered rotation of the imidazole base around the iron–nitrogen bond and van der Waals attraction between the ortho groups and the ligand have also been considered.

It is of some importance to understand the nature and magnitude of such ortho effects in making comparisons of the binding and catalytic behavior of the model systems with those of heme proteins.[22,26] We have therefore investigated binding equilibria and kinetics of ligation to the iron(II) derivatives of tetraphenyl porphyrin (FeTPP) and tetramesityl porphyrin (FeTMP).

To address the solvation idea we have compared the ortho effect (i.e. FeTPP vs FeTMP) on binding to four and five coordinated iron porphyrins (eq 7 and 8), as well as on the displacement equilibria (eq 9).



(7)

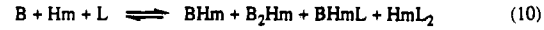
(8)

(9)

The solvation of FeTPP should have the same effect upon eq 7 and 8 since solvation of both sides of four coordinated iron porphyrins should reduce affinities to the same extent as in the case of the five coordinated heme. By contrast, since there is no open metal site in the displacement equilibrium of eq 9, the solvation effect should be absent.

It is expected that any effect which resists doming would retard dissociation rates which lead to the usually doomed five coordinated complexes. It is therefore of some interest to determine the ortho effects on the dissociation and association rates as well as the equilibrium constants.

We report both kinds of studies and include some novel methods of determining the individual kinetic constants involved in the interactions of a mixture of an iron(II) porphyrin with two different ligands.



(10)

Experimental Section

Reagents. Toluene (Mallinckrodt AR) was distilled under an argon atmosphere from sodium benzophenone ketyl and stored over activated molecular sieves (4 Å, MCB). 1-Methylimidazole (MI) (Aldrich) was distilled under reduced pressure from calcium hydride and stored over activated molecular sieves (4 Å, MCB). 1,2-Dimethylimidazole (DMI) (Aldrich) was dried over KOH, distilled under vacuum, and stored below 0 °C. Tosylmethylisocyanide (TMIC, Aldrich), benzophenone (Aldrich), 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6, Fluka), and methanol (Mallinckrodt spectra grade) were used without further purification. Sodium hydrosulfite (sodium dithionite, Virginia Chemical Co.) was stored in small Teflon-capped vials at 4 °C until use. Argon gas (Linde Products, commercial grade) was passed through an oxygen scrubber (American Scientific Products) prior to use. Carbon monoxide gas (Matheson, 99.99%) was used as received.

meso-Tetraphenylhemin chloride (Fe^{III}TPPCl), (Fe(II) form, TPPFe) and *meso*-tetramesitylhemin chloride (Fe^{III}TMPCl), (Fe(II) form, TMPFe) were prepared by literature procedures.[27]

Instrumentation. The instruments used, the computer interfaced Kontron Uvikon 810 spectrophotometer, the microsecond, nanosecond, and sub-picosecond laser kinetic instruments have been described elsewhere.[28,29]

Sample Preparation for Titration Experiments. Two methods were used to reduce the hemin samples prior to addition of the appropriate ligands. For experiments involving the titration of an iron porphyrin complex with CO, the solutions were reduced by the photochemical method of Chang.[30] When zero CO concentrations were required, this method could not be used. In these cases, hemin solutions in toluene containing base or TMIC were deoxygenated by passing toluene saturated argon gas through it for 1 h. Then aliquots of 1–2 μL of crown ether-dithionite complex in methanol (CDM solution) were added as previously described.[31]

Spectrophotometric Titrations. Solutions of reduced hemes, TPPFe and TMPFe, in toluene were prepared by one of the above methods. In order to determine equilibrium constants, titrations were performed

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spectrophotometrically, following equilibration at 20.0 ± 0.1 °C. The heme-base-CO complexes were prepared using 0.7 M 1-methylimidazole (MI) or 0.3 M 1,2-dimethylimidazole (DMI). Solutions of 0.1 M MI and 1M DMI were made for the bis-base complexes. The bis-TMIC complexes were prepared using solutions of 1 mM or 10 mM TMIC depending upon the titration be done with MI or DMI respectively. Ligands were added via gastight syringe as toluene solutions. Clean isosbestic points were obtained in the visible spectra after corrections for dilutions were made.

Sample Preparation for Measurement of Carbon Monoxide Dissociation Rate Constants. The heme solutions were reduced to the ferrous state in a specially designed tonometer[32] that was constructed in such a way as to allow complete evacuation and addition of argon to ensure anaerobic conditions and this tonometer was used as previously described.[32]

A concentrated solution of hemin chloride in toluene was added via syringe to 3 mL of 0.5 M or 1 M the imidazole (MI or DMI) solution in toluene, to yield a hemin concentration of $\sim 10^{-6}$ M. The sample was sealed in a tonometer, and the entire cell deoxygenated by passing toluene saturated carbon monoxide gas through it for 30 min. Next, the hemin was reduced with 2–3 μL of CDM solution, as described above.

With the tonometer held horizontally, the heme solution was transferred to the bulb section containing a small stir bar. Then the solution was degassed with three freeze–pump–thaw cycles and the tonometer filled with argon.

Aliquots of carbon monoxide were added to the tonometer by a gastight syringe inserted into the septum in the stopcock. After each CO addition the septum was replaced and the space between it and the stopcock flushed with argon.

Sample Preparation for Measurement of Second-Order Rate Constants, k_B^B. The sample was first prepared in a small 1-cm square cell, under 1 atm of CO, using a CDM solutions as reducing agent and 10^{-5} M heme, as described above. A 2-mm quartz cuvette was sealed with a silicon rubber septum and flushed with argon for 30 min. Then the base–Heme–CO solution was transferred to the 2-mm cuvette under CO atmosphere with a cannula.

The K^B and K_B^B values for TPPFe[33] and TMPFe[34] were used to calculate MI or DMI concentrations that give greater than 99% of HmB_2 and ~0% of four-coordinated species after photolysis of the carbon monoxide complex.

Sample Preparation for Direct Measurement of Carbon Monoxide Association Rate Constants. All kinetic runs for direct measurements of k_B^{CO} were performed in a specially designed high-pressure cell shown in Figure 1.

The sample was first prepared in a 1-cm square cell, under 1 atm of carbon monoxide, and reduced as described above. The 10^{-5} M heme solution was added via a gastight syringe to the high pressure cell (Figure 1), previously deoxygenated with carbon monoxide. A CO pressure of 200 psi was admitted into the cell for 15–20 min and then released to ~20 psi. This procedure was repeated 3 times to make sure all oxygen was removed. The desired carbon monoxide pressure was added and the UV–visible spectrum was recorded, showing the characteristic Soret of BHmCO species.

Results

Titrations. The titration of tetraphenylheme–dimethylimidazole–CO complex with tosylmethylisocyanide (TMIC) is shown in Figure 2 as a typical example of the titration method. Figure 3 shows plots of $^1/(OD_O - OD)$ versus CO/TMIC from which the equilibrium constant can be calculated. Equations defining this and the several other titrations along with other equilibria, established in other ways are given in Table I.

The values of the constants for these equilibria are given in Table II. Results of direct titrations are given without reference. Other values which have been calculated from ratios of experimental titration constants or by kinetic methods are noted with footnotes.

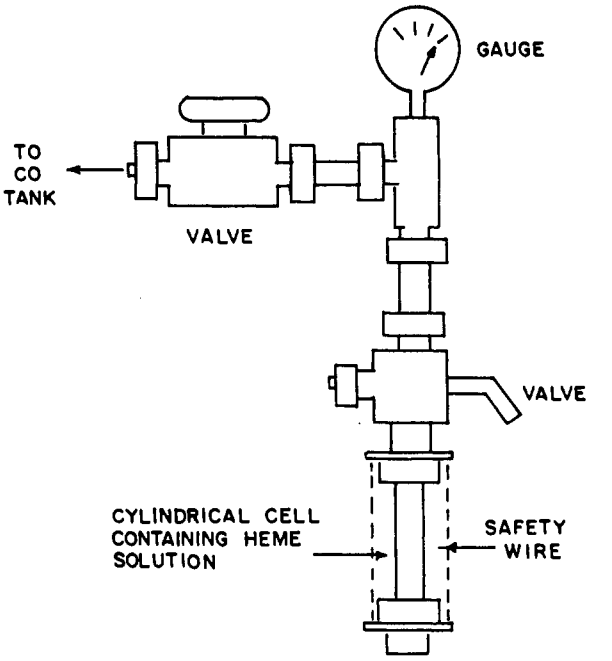

Figure 1. High-pressure cell for direct measurement of k_B^{CO}. All materials are stainless steel except for the cell which is Pyrex glass, $^1/_4$-in. o.d.

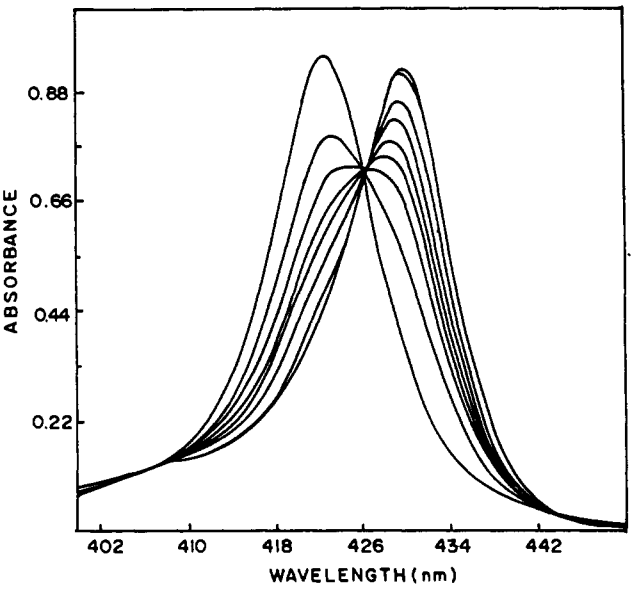

Figure 2. Titration of TPPFe(DMI)CO with TMIC in toluene at 20 °C. The increasing in absorbance at 431 nm corresponds to the following concentrations of TMIC (× 10^4) (M): 0, 1.0, 2.0, 3.0, 3.9, 5.9, 8.8, 12.6, 17.3, and 25.5. [heme] = 3 × 10^{-6} M, [CO] = 7.8 × 10^{-3} M, $[TMIC]_{1/2}$ = 2.6 × 10^{-4} M, $K_{DMI}^{CO,TMIC}$ = 30 ± 0.8.

In several cases the constants have been determined in two ways or have also been reported elsewhere.

Kinetics. Three different instruments were required to obtain the desired kinetic constants. At high base concentration the rate of addition of CO is very slow due to the formation of HmB_2.

$$BHmCO \underset{k_B^{CO}}{\overset{h\nu}{\rightleftharpoons}} BHm + CO \overset{K_B^B}{\rightleftharpoons} B_2Hm \qquad (11)$$

Therefore, the rate appears as a pre-equilibrium followed by rate-limiting addition of carbon monoxide (eq 12). At 25° the concentration of CO is 10^{-5} Torr.[32] First-order decays were always

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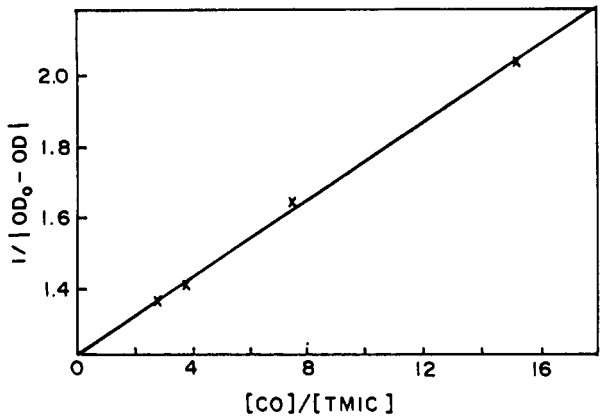


Figure 3. Plot of $1/|OD_0 - OD|$ against [CO]/[TMIC] for the titration of TPP(MI)CO with TMIC in toluene at 20 °C, using 0.7 M MI. TPPFe [heme] = 4×10^{-6} M; [CO] = 7.3×10^{-3} M, $[TMIC]_{1/2} = 3.2 \times 10^{-4}$ M. Optical densities were monitored at 423 nm. Intercept = 1.22 ± 0.01, slope = 0.054 ± 0.001, $r = 0.999$, $K_{MI}^{CO,TMIC}$ = intercept/slope = 22.6 ± 0.04.

Table I. Equilibria for Binding and Replacement Reactions[a]

E1	$Hm(MI)(TMIC) + CO \overset{K_{MI}^{TMIC,CO}}{\rightleftharpoons} Hm(MI)CO + TMIC$
E2	$Hm(DMI)(TMIC) + CO \overset{K_{DMI}^{TMIC,CO}}{\rightleftharpoons} Hm(DMI)CO + TMIC$
E3	$Hm(MI)(n\text{-}BuNC) + CO \overset{K_{MI}^{n\text{-}BuNC,CO}}{\rightleftharpoons} Hm(MI)CO + n\text{-}BuNC$
E4	$Hm(MI)(TMIC) + MI \overset{K_{MI}^{TMIC,MI}}{\rightleftharpoons} Hm(MI)_2 + TMIC$
E5	$Hm(DMI)(TMIC) + DMI \overset{K_{DMI}^{TMIC,DMI}}{\rightleftharpoons} Hm(DMI)_2 + TMIC$
E6	$Hm(TMIC)_2 + MI \overset{K_{TMIC}^{TMIC,MI}}{\rightleftharpoons} Hm(TMIC)(MI) + TMIC$
E7	$Hm(TMIC)_2 + DMI \overset{K_{TMIC}^{TMIC,DMI}}{\rightleftharpoons} Hm(TMIC)(DMI) + TMIC$
E8	$Hm(MI)_2 + CO \overset{K_{MI}^{MI,CO}}{\rightleftharpoons} Hm(MI)CO + MI$
E9	$Hm(DMI)CO + DMI \overset{K_{DMI}^{CO,DMI}}{\rightleftharpoons} Hm(DMI)_2 + CO$
E10	$Hm(MI) + TMIC \overset{K_{MI}^{TMIC}}{\rightleftharpoons} Hm(MI)(TMIC)$
E11	$Hm(DMI) + TMIC \overset{K_{DMI}^{TMIC}}{\rightleftharpoons} Hm(DMI)(TMIC)$
E12	$Hm(MI) + CO \overset{K_{MI}^{CO}}{\rightleftharpoons} Hm(MI)CO$
E13	$Hm(MI) + MI \overset{K_{MI}^{MI}}{\rightleftharpoons} Hm(MI)_2$
E14	$Hm(DMI) + CO \overset{K_{DMI}^{CO}}{\rightleftharpoons} Hm(DMI)CO$
E15	$Hm(DMI) + DMI \overset{K_{DMI}^{DMI}}{\rightleftharpoons} Hm(DMI)_2$

[a] See the Experimental Section and eq 5 for abbreviations used here and in the following tables.

observed. A typical plot of the observed rate constant against CO pressure, P_{CO}, is given in Figure 4. Such rates can be measured on a spectrophotometer.

$$k_{obs} = \frac{k_B^{CO} \times 10^5}{1 + K_B^B(B)} P_{CO} + k_B^{CO} \qquad (12)$$

In order to obtain k_B^{CO} directly, a nanosecond laser is required. The dissociation of the five-coordinate species or the formation of B_2Hm would otherwise interfere. The measured rate must be greatly in excess of 10^3 s^{-1} in order to avoid the base off mechanism (eq 13).[31] Therefore high pressures of CO and fast rate

Table II. Equilibrium Constants for Tetramesitylheme and Tetraphenylheme in Toluene at 20 °C

eq	equil consts	hemes: K(TMPFe)[a]	hemes: K(TPPFe)[a]	ratio K(TMPFe)/K(TPPFe)
E1	$K_{MI}^{TMIC,CO}$	$(6.4 \pm 0.2) \times 10^{-1}$	$(4.44 \pm 0.09) \times 10^{-2}$	14
E2	$K_{DMI}^{TMIC,CO}$	$(2.8 \pm 0.2) \times 10^{-1}$	$(3.33 \pm 0.09) \times 10^{-2}$	8
E3	$K_{MI}^{n\text{-}BuNC,CO}$	1.9 ± 0.1	$(5.8 \pm 0.2) \times 10^{-1}$	3
E4	$K_{MI}^{TMIC,MI}$	1.6×10^{-4} *(a)* 1.9×10^{-4} *(b)*	2.4×10^{-5} *(b)*	8
E5	$K_{DMI}^{TMIC,DMI}$	2.9×10^{-5} *(a)* 1.7×10^{-5} *(b)*	9.1×10^{-8} *(b)*	320 186
E6	$K_{TMIC}^{MI,MI}$	38 ± 3	$(5.2 \pm 0.5) \times 10^{-1}$	73
E7	$K_{TMIC}^{TMIC,DMI}$	1.20 ± 0.05	$(8.5 \pm 0.9) \times 10^{-3}$	141
E8	$K_{MI}^{MI,CO}$	3.3×10^3 *(b)*	1.9×10^3 *(b)*	2
E9	$K_{DMI}^{CO,DMI}$	6.0×10^{-5} *(b)*	2.6×10^{-6} *(b)*	23
E10	K_{MI}^{TMIC}	4.8×10^8 *(b)*	3.3×10^9 *(b)*	0.1
E11	K_{DMI}^{TMIC}	5.0×10^7 *(b)*	2.7×10^7 *(b)*	2
E12	K_{MI}^{CO}	3.02×10^8 *(c)*	1.47×10^8 *(c)*	2
E13	K_{MI}^{MI}	9.0×10^4 *(d)*	7.8×10^4 *(d,e)*	1
E14	K_{DMI}^{CO}	1.4×10^7 *(c)*	9.1×10^5 *(c)*	15
E15	K_{DMI}^{DMI}	8.4×10^2 *(d,e)*	2.45 *(f)*	340

[a] All data were determined directly by titration except as noted, units are those appropriate to equilibria 1–15 of Table I. [b] Calculated from other equilibria. [c] Calculated from kinetic data. [d] Calculated from k_B^{CO} at constant B. [e] Calculated from kinetic and equilibria data. A similar value was obtained by direct titration. [f] From ref 33a.

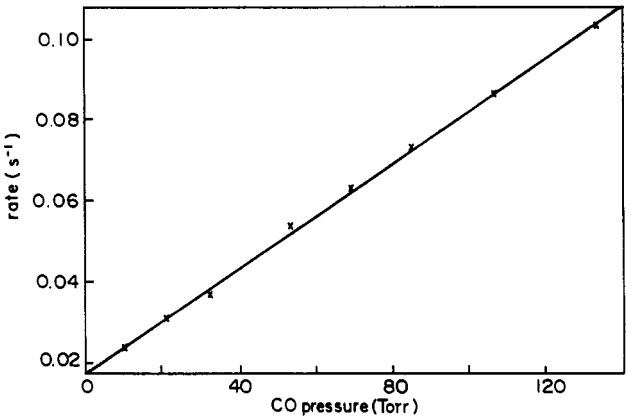
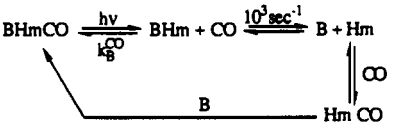


Figure 4. Plot of k_{obsd} versus carbon monoxide partial pressure over toluene at 20 °C for 10^{-6} M, TPPFe in 0.5 M MI. Slope = $(6.48 \pm 0.09) \times 10^{-4}$ Torr^{-1} s^{-1}, intercept = $k_B^{CO} = (1.71 \pm 0.06) \times 10^{-2}$ s^{-1}, $r = 0.999$.

measurements are required and the high-pressure cell shown in Figure 1 was used. A plot of absorbance versus time is shown in Figure 5. This curve is accurately exponential.



(13)

An even more difficult association rate measurement was encountered in the binding of 1,2-dimethylimidazole to TPPFe where the equilibrium constant for binding the second base is

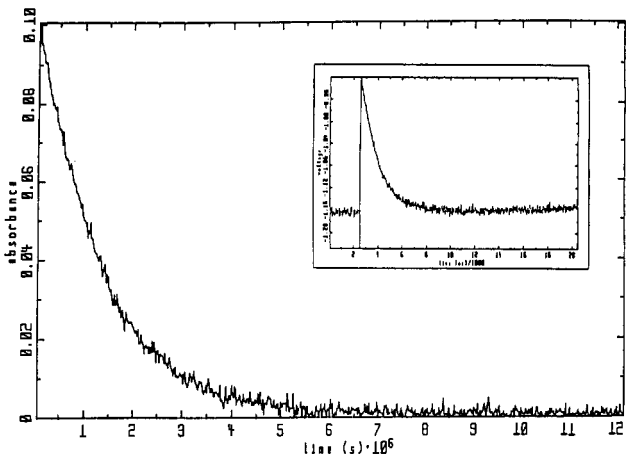


Figure 5. Absorbance vs time and voltage vs time plots obtained during measurements of k_B^{CO} for TMPFe(MI)CO in the nanosecond flash photolysis apparatus under 15.36 atm of CO. Photolysis energy was 3 mJ at 540 nm. [heme] = 10^{-5} M; [MI] = 10^{-4} M.

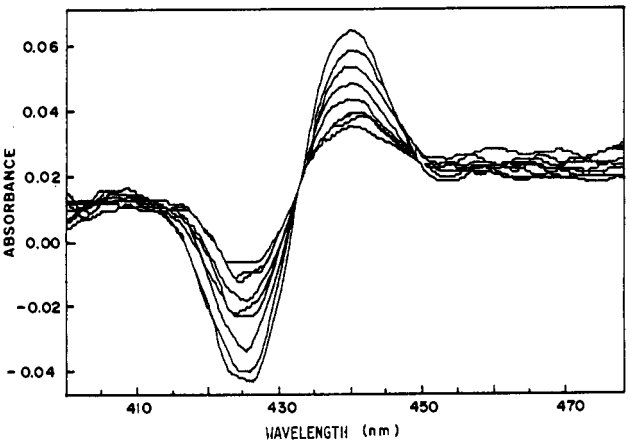


Figure 6. Picosecond transient difference spectra of TPPFe(DMI)CO in toluene under 1 atm of CO and 2.99 M DMI. Successive traces were taken at 400, 750, 1000, 1250, 1500, 1750, 2000, and 2250 ps. Photolysis energy was 60 μJ at 314 nm. [heme] = 10^{-5} M.

only 2.5.[33a] In this case it is necessary to use 3 M 1,2-DMI in order to form the bis-1,2-DMI complex.

$$\text{1,2 DMI-HmCO} \xrightleftharpoons{h\nu} \text{1,2 DMI-Hm} + \text{CO} \xrightleftharpoons{k_B^B} (\text{1,2-DMI})_2\text{Hm} \quad (14)$$

This increases the pseudo-first-order rate constant to about 10^9 s^{-1} which cannot be measured with our 4-ns laser system. Therefore photolysis was carried out with the ps laser (described elsewhere).[29] A series of spectra after photolysis of the DMI-(TPPFe)CO complex are shown in Figure 6. Although the infinity point is not reached at the time limit of the laser system (~2000 ps) the rate constant can be estimated. The difference spectra corresponds closely to HmB and HmB_2. From the known equilibrium constant $K_B^B = k_B^B/k_B^{-B} = 2.5$, and the equation for the observed rate constant, $k_{obs} = k_B^B(B) + k_B^{-B}$, the rate constants $k_B^B = 2.4 \times 10^8$ M^{-1} s^{-1} and $k_B^{-B} = 10^8$ s^{-1} can be calculated.

The rate constants are shown in Table III along with published values for myoglobin and hemoglobin.

Discussion

Before discussing the ortho effect it is instructive to consider the "T-state" effect,[1] that is the effect of replacing 1-methylimidazole (MI) with 1,2-dimethylimidazole (DMI), an effect already attributed to a steric resistance of the latter base to the planarity demanded by six-coordinated heme.[3,38] As previously reported, this replacement lowers the affinity of BHm for CO by about 150-fold. The equilibria calculated from Table I can be used as a measure of this effect on the following equilibria. The effects are given as

$$\Delta K = (K)_{B=MI} / (K)_{B=DMI} \quad (15)$$

for the indicated equilibrium.

$$\begin{array}{ccccc} \text{BTPPFe} + \text{CO} & \underset{(a)}{\overset{\Delta K=170}{\rightleftharpoons}} & \text{BTPPFe CO} & \underset{(b)}{\overset{\Delta K\cong 150}{\rightleftharpoons}} & \text{B} + \text{TPPFeCO} \\ & & \Delta K=130 \Big\| \text{(c)} & & \\ & & \text{B}_2\text{TPPFe} & & \end{array} \quad (16)$$

Whether BHmCO is assembled from a five-coordinated BTPPFe or a five-coordinated TPPFeCO the steric effect of 1,-2DMI is about the same. This derives from the approximately equal affinities (K_{17}) of MI and DMI for four-coordinated heme.[33ab,35,39-41]

$$\text{TPPFe} + \text{B} \xrightleftharpoons{K_{17}} \text{BTPPFe} \quad (17)$$

Notice that an additional steric effect of about 130 accrues upon replacing CO with a second base. In this case DMI favors the CO complex as a result of the severe repulsion of two DMI molecules. This additivity is seen in the following equilibrium:

$$\text{BTPPFe} + \text{B} \xrightleftharpoons{\Delta K=3\times10^4} \text{BTPPFeB} \quad (18)$$

Thus none of the ("T-state") steric effect appears in the addition of the first base, the entire effect appearing in the second step.

An entirely similar situation is displayed in the equilibria involving the rather electronegative isocyanide, tosylmethylisocyanide (TMIC).

$$\begin{array}{ccccc} \text{BTPPFe} + \text{TMIC} & \underset{(a)}{\overset{\Delta K=120}{\rightleftharpoons}} & \text{BTPPFeTMIC} & \underset{(b)}{\overset{60}{\rightleftharpoons}} & (\text{TMIC})_2\text{TPPFe} \\ & & \Delta K=250 \Big\| \text{(c)} & & \\ & & \text{B}_2\text{TPPFe} & & \end{array} \quad (19)$$

That the "T-state" effect is present in the binding of both CO, TMIC and imidazoles (as well as O_2)[38] shows the generality of this steric repulsion and confirms the suggestion that these ligands require heme planarity in the six-coordinated state. It is interesting that the effects of replacing the first and second unhindered ligand with DMI do not differ very much (60–250) and the addition of B to B(TPPFe) shows a ΔK which is energetically about equal to the sum of the replacement energies for the first and second ligand with B. The repulsion is around 3 kcal/M per DMI for all situations.

Although TMPFe systems are quantitatively different from those of TPPFe, the "T state" effect remains. For example, the ΔK for CO affinities to TMPFe complex with MI and DMI is 22. The reduction in the size of the effect introduces the subject of ortho substitution on TPP derivatives.

The Ortho Effect. The effect of ortho substituents on ligation equilibria was clearly seen in the affinities of TPPFe and TMPFe

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(37) (a) MacQuarrie, R.; Gibson, Q. H. *J. Biol. Chem.* **1971**, *246*, 5832–5835. (b) Gibson, Q. H. *Prog. React. Kinet*; Porter, G., Ed.; Macmillan Co.: New York, 1964; Vol. 2; p 328.
(38) Collman, J. P. *Acc. Chem. Res.* **1977**, *10*, 265–272.
(39) Dr. Francis Knowles, Unpublished data. K^{DMI} = 1.4 × 10^4 M^{-1} for TMPFe in toluene.
(40) White, D. K.; Cannon, J. B.; Traylor, T. G. *J. Am. Chem. Soc.* **1979**, *101*, 2443–2454.
(41) (a) Brault, D.; Rougee, M. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 654–659. (b) Rougee, M.; Brault, D. *Biochemistry* **1975**, *14*, 4100–4106. (c) Brault, D.; Rougee, M. *Biochemistry* **1974**, *13*, 4591–4597.

Table III. Equilibrium and Rate Constants for CO and Imidazole Binding to Tetraphenylheme, Tetramesitylheme, and Hemoproteins at 20 °C

heme (B)	k_B^{-CO} (s^{-1})	k_B^{CO} (M^{-1} s^{-1})	K_B^{CO} (M^{-1})	k_B^B (M^{-1} s^{-1})	K_B^B (M^{-1})	k_B^{-B} (s^{-1})
TPH (MI)	0.0171 ± 0.0006	(2.52 ± 0.05) × 10^6 2.48 × 10^6 (*a*)	1.47 × 10^8	(1.32 ± 0.05) × 10^8 1.6 × 10^8 (*b*)	7.8 × 10^4 1.4 × 10^5 (*c*)	1.7 × 10^3
TMH (MI)	0.0109 ± 0.0005	(3.29 ± 0.06) × 10^6	3.02 × 10^8	(1.18 ± 0.02) × 10^8	9.0 × 10^4	1.3 × 10^3
$FePiv_35CIM$[d]	0.0078	3.6 × 10^7	4.5 × 10^9			
TPH (DMI)	0.09 ± 0.03 0.24[e]	8.2 × 10^4 1.6 × 10^5 (*d*)	9.10 × 10^5 6.70 × 10^5 (*f*) 7.10 × 10^5 (*g*)	2.4 × 10^8	2.45[g]	1 × 10^8
TMH (DMI)	0.06 ± 0.01	(8.43 ± 0.03) × 10^5	1.40 × 10^7 1.30 × 10^7 (*b*)	(1.36 ± 0.05) × 10^7	8.4 × 10^2	1.6 × 10^4
FeTPivP (DMI)[d]	0.14	1.4 × 10^6	1.1 × 10^7			
horse Mb[h]	0.017	5.0 × 10^5	2.9 × 10^7			
R-state Hb[i]	0.009	6 × 10^6	7 × 10^8			
T-state Hb[i]	0.09	1 × 10^5	1 × 10^6			

[a] From ref 34, after we correct their value using our K_{MI}^{MI} value in their data: $K_B^{CO} = 31.8K_B^B$ (from their Table I). In their calculation, they had assumed $K_B^B = 10K^B$ to obtain K_B^B from the ratio $K_B^BK^B = 3.33 \times 10^8$ M^{-2}. Their published value was $k_{MI}^{CO} = 1.8 \times 10^6$ M^{-1} s^{-1}. [b] 25 °C, from ref 35. [c] In benzene, from ref 33b. [d] From ref 23. [e] Calculated from $P_{1/2}$ and K_B^{CO}, 25 °C; from ref 23. [f] Measured with a tonometer at 25 °C; from ref 23. [g] From ref 33a. [h] From ref 1. [i] k_B^{-CO} from ref 36. [j] k_B^{CO} from ref 37.

for two DMI ligands, the difference appearing entirely in the second step.

$$\text{DMI(TPPFe)} + \text{DMI} \underset{}{\overset{K=2.45}{\rightleftharpoons}} \text{TPPFe(DMI)}_2 \qquad (20)$$

$$\text{DMI(TMPFe)} + \text{DMI} \underset{}{\overset{K=840}{\rightleftharpoons}} \text{TMPFe(DMI)}_2 \qquad (21)$$

The striking difference between TMPFe and TPPFe can be seen in comparison of the effects of DMI on the equilibria discussed for TPPFe above. The number, $\Delta K \simeq 20$ for (22b) has been

$$\begin{array}{ccccc} \text{BTMPFe} + \text{CO} & \overset{\Delta K=22}{\underset{\text{(a)}}{\rightleftharpoons}} & \text{B(TMPFe)CO} & \overset{\Delta K=20}{\underset{\text{(b)}}{\rightleftharpoons}} & \text{B} + \text{TMPFeCO} \\ & & \Delta K=5 \Big\uparrow\Big\downarrow \text{(c)} & & \\ & & (\text{TMPFe})\text{B}_2 & & \end{array} \qquad (22)$$

calculated based upon the general observation that the equilibrium constants for adding imidazoles and CO to four-coordinated hemes are approximately equal ($K \simeq 10^4$ M^{-1}).[42] This results in $K_B^{CO} \simeq K_{CO}^B$ from the relationships among these equilibria. The backbonding trans effect is about the same in both directions.

$$\begin{array}{ccccc} \text{BTMPFe} + \text{TMIC} & \overset{\Delta K=9.5}{\underset{\text{(a)}}{\rightleftharpoons}} & \text{B(TMPFe)TMIC} & \overset{}{\underset{\text{(b)}}{\rightleftharpoons}} & \text{B} + (\text{TMPFe})\text{TMC} \\ & & \Delta K=5.5 \Big\uparrow\Big\downarrow \text{(c)} & & \\ & & (\text{TMPFe})\text{B}_2 & & \end{array} \qquad (23)$$

The T-state effect is drastically reduced in the TMPFe series, in one case from 260 to 5.5. This reduction in the T-state effect is found for both CO and TMIC. Since TMIC has a phenyl group which is capable of van der Waals interaction with the TMP mesityl groups this observation argues against such attractions being involved. When both steric effects are introduced at once as in eqs 18 and 24, the ΔK for DMI replacing MI is reduced from 3 × 10^4 (TPPFe) to 100 (TMPFe).

$$\text{B(TMPFe)} + \text{B} \underset{}{\overset{\Delta K=100}{\rightleftharpoons}} (\text{TMPFe})\text{B}_2 \qquad (24)$$

These data agree with published values of the CO affinities of ortho substituted tetraphenylhemes. The med pocket,[23] C_2 cap and C_3 cap[33a,43] all have ΔK for CO addition around 40. Hemes such as TPPFe, or deuteroheme which do not have this ortho substitution have values around 150. There is one exception to this observation, the picket fence hemes have $\Delta K = 400$ for this process,[23] larger than even the unsubstituted hemes and completely out of line with all other ortho substituted hemes. We have no explanation for this. It is not at all clear why the effect of DMI vs MI on the CO affinity should be 400 for the tetrapivalyl system and only 40 for the similar med pocket heme. It is remarkable that three ortho substituted hemes, picket fence heme–DMI,[23] tetrakis(2,6-diphenylphenyl)heme-DMI,[44] and tetramesitylheme–DMI have almost identical half pressures for CO binding ($P_{1/2}^{CO}$ = 0.009–0.007 Torr) and yet chelated picket fence heme (an unstrained five-coordinated heme) binds CO thirty times more strongly than does tetramesitylheme–1-MeIm.[23] Similarly high CO affinities have been reported[6] for the series of "basket handle hemes" which resemble the picket fence heme in having ortho substituted phenyl groups and they are not "T-state" models such as those employing DMI as a proximal base. Our results with TMPFe duplicate the T-state model binding of picket fence heme but not the R-state behavior of either basket handle or picket fence heme. Some other factors must be involved in the latter two systems.

Addressing only the data presented here and the published T-state data, we can conclude that ortho substituents increase the affinities for ligands even though they should be more sterically hindered. Furthermore, there is a strong coupling between the T-state and ortho effects. The T-state equilibria are changed more then are R-state models. For example K_{DMI}^{DMI} is 15 times larger for TMPFe than for TPPFe and the replacement equilibrium constant, $K_{DMI}^{TMIC,DMI}$, is 300 times larger than TMPFe than for TPPFe. On the other hand, K_{MI}^{MI} is about the same for the two hemes and K_{MI}^{CO} is only twice as large for TMPFe as for TPPFe. The ortho effect is clearly larger for T-state systems, i.e. those involving DMI and therefore those in which there is hindrance to planarity. It therefore seems reasonable to assign this increase in affinity in the T-state models to a cancellation of the T-state effect.

Although the T-state and ortho effects are strongly coupled, the T-state effect and distal steric effect act independently. Thus the essentially unhindered 7,7-anthracenecyclophane heme and the highly hindered 6,6-anthracenecyclophane heme have the same T-state effect.[45] Picosecond kinetic studies of cyclophane hemes have shown that the distal steric effect occurs prior to the bond making step whereas the T-state effect is in the bond making (or breaking) step.[46] Therefore these effects shoiuld act independently.

(42) Rougee, M.; Brault, D. *Biochem. Biophys. Res. Commun.* **1973**, *55*, 1364–1369.
(43) (a) Linard, J. E.; Ellis, P. E., Jr.; Budge, J. R.; Jones, R. D.; Basolo, F. *J. Am. Chem. Soc.* **1980**, *102*, 1896–1904. (b) Rose, E. J.; Venkatasubramanian, P. N.; Swartz, J. C.; Jones, R. D.; Basolo, F.; Hoffman, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5742–5745.
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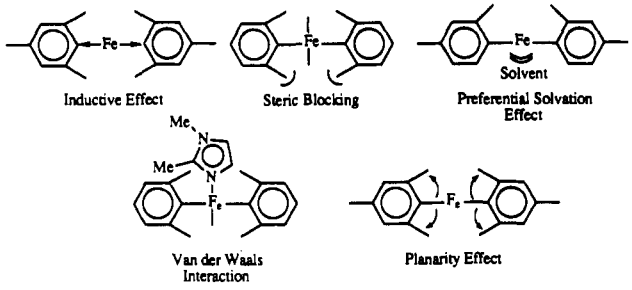
(45) Traylor, T. G.; Tsuchiya, S.; Campbell, D.; Mitchell, M.; Stynes, D.; Koga, N. *J. Am. Chem. Soc.* **1985**, *107*, 604–614.
(46) Traylor, T. G.; Taube, D. J.; Jongeward, K. A.; Magde, D. *J. Am. Chem. Soc.* **1990**, *112*, 6875–6880.

That the ortho effect changes dissociation rates and correlates with the T-state effect provides evidence that this effect operates at the bond-making step.

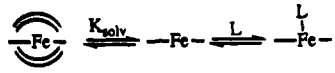
The Nature of the Ortho EFfect. This effect could accrue from 1) an electronic effect of the phenyl group; 2) steric blocking of the ligand by the ortho group; 3) a solvation of the "flat" heme (TPPFe) which is sterically prevented in the ortho substituted heme; 4) an attractive van der Waals interaction with the ligand, e.g. DMI; or 5) a conformational effect in which the ortho group reduces doming, keeping the ring more planar.



Inductive Effects. The ortho-methyl substituents prevent the phenyl rings from attaining planarity with the porphyrin thus reducing resonance interaction. The result is a possible increase in electron withdrawing effect of the phenyl group. This would result in stronger binding of electron rich ligands to TMPFe. This is not generally the case. Whereas the more electronegative TMIC is replaced by DMI much more effectively in the case of TMPFe (E7) the more electronegative carbon monoxide is preferred over *n*-BuNC by TMPFe (E3). There is no consistent inductive effect displayed.

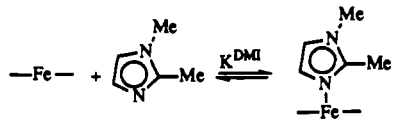
Steric Blocking of Ligands. Although steric effects of ortho substituents on the selectivities in epoxidations catalyzed by these hemin catalysts are well documented,[13,24] there is no evidence for preferential binding of smaller ligands in this study. For example, the replacement of the smaller carbon monoxide ligand by 1,2-dimethylimidazole (E9) is *favored* by the more blocked TMPFe. The same effect with 1-methylimidazole is smaller (E8). The preference of TMPFe for 1,2-dimethylimidazole is even more strikingly shown in a comparison of E13 with E15. Steric effects on these ligands seems to be minimal and cannot account for the ortho effect.

Solvation of Flat Hemes. This suggestion, made to explain the stronger CO binding to picket fence heme,[38] assumes that tetraphenylheme, chelated protoheme, etc., not bearing the protecting ortho groups, would be solvated strongly over the face of the iron, reducing ligation.



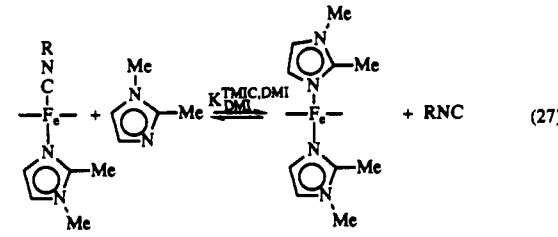
(25)

Since ortho substituents would interfere with this solvation, shifting the equilibrium K_{solv} to the right, such ortho substitution should increase the overall equilibrium constant K^L. Two kinds of experimental facts are inconsistent with this idea. First, the binding constants (K^{DMI}) for addition of the first 1,2-dimethylimidazole to TMPFe and TPPFe are 1.3×10^4 and 2.7×10^4 M^{-1}.[33a]



(26)

But more to the point, the ortho effect (K_{TMH}/K_{TPH}), is almost as large for the replacement of TMIC by 1,2-dimethylimidazole (E5) as it is for the addition of a second 1,2-DMI to the five-coordinated heme (E15).

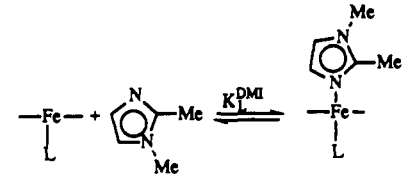


(27)

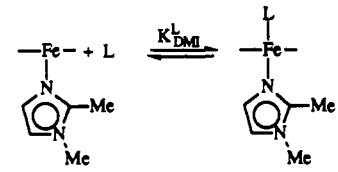
Since no five-coordinated form is involved, these results cannot be explained by a preferential solvation of the deoxy form of a flat heme.

van der Waals Attraction. After this work was finished,[47] the binding of 1,2-dimethylimiazoles to the ferric forms of tetraphenyl- and tetramesitylporphyrins was reported by Nakamura.[48] Finding an ortho effect in these equilibria these authors suggested that these might be some attractive interaction between the DMI and the methyl groups. This would not explain the ortho effect on carbon monoxide binding (E14) or the replacement of TMIC with 1-methylimidazole (E6) or with CO (E1). While it is true that the largest effects of the ortho substituents are in the addition of (E15) or replacement by (E5,E7) 1,2-dimethylimidazole, the ortho effect is seen in other displacements. This could as easily be attributed to the "T-state nature" of the ligand. Thus, a comparison of the K_B^{CO} for TPPFe in E12 vs E14 reveals a 160-fold reduction of this equilibrium constant upon changing B from 1-methylimidazole to 1,2-DMI. We therefore do not find the van der Waals attraction to be completely consistent with our results. By either dividing K_{E6} by K_{E7} or K_{E10} by K_{E11} the binding of methylimidazole versus 1,2-dimethylimidazole to HmTMIC can be estimated. This reveals that MI binds 60 to 100 times better than does DMI to TPPFe but only 10–30 times better for TMPFe. This is another example of a planarity effect, reducing the difference between DMI and MI discussed below, and it is not consistent with a van der Waals attraction. Finally, the tosylmethyl isocyanide equilibrium $K_B^{TMIC,CO}$ should favor TMIC with TMPFe and it does not (E1 and E2).

Planarity Effect. The most striking effect in Table I is the large ortho effect which results when DMI is either the entering ligand or the "spectator ligand."



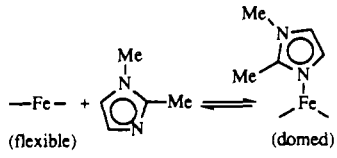
(28)



(29)

The replacement of 1-methylimidazole with 1,2-dimethylimidazole as the "spectator" ligand in E10 vs E11 and E12 vs E14 demonstrates again the well known[3,38] T-state effect which is generally attributed to repulsions between the 2-methyl group and the heme plane. The four coordinated heme is apparently quite flexible and thus the addition of the first DMI does not encounter steric hindrance.

(47) Portela, C. Thesis, University of California, San Diego, CA, 1987.
(48) Nakamura, M. *Inorg. Chim. Acta.* **1989**, *161*, 73–80.



(30)

By contrast, when a ligand is already present and the addition of DMI enforces planarity, severe steric hindrance is encountered (compare E6 with E7). The most striking evidence for a close relationship between the ortho effect and the T-state effect is seen in the comparison (E13 and E15). In E13 where no T-state structures are involved TMPFe and TPPFe bind 1-MeIm with the same affinity.

The effects of steric hindrance (T-state effect) is reduced when the heme is TMPFe. We therefore suggest that TMPFe reduces the T-state effect by hindering the porphyrin doming. The TMPFe type hemes seem to be much less flexible than are other hemes.

Recently[49] various bispyridine TMPFe[III] complexes have been studied in detail by x-ray crystallography, electron spin resonance, nuclear magnetic resonance and Mössbauer spectroscopy. The crystal structures showed a ruffled configuration in which opposite methine positions were tilted up (two) and down (two) by $\pm\sim 0.35$ Å. This ruffling has electronic consequences which strongly affect spectroscopic properties and could affect binding as well. In the absence of structural data we have used the term "stabilization of planarity" to explain the stronger binding to BTMPFe. The ruffled structure found for the iron(III) complexes was attributed to the effects of tilting the mesityl groups. Our interpretation is consistent with a ruffled rather than planar structure. The energy required to convert the ruffled structure to a domed five-coordinated structure would then explain the resistance to doming.

While the picket fence binding of CO has the imidazole on the side opposite to the ortho groups and would preclude an effect of rotation about the iron–imidazole bond as being responsible for the ortho effect the special structure of TMPFe, whether planar or ruffled accounts for the special binding properties associated with the ortho effect.

Kinetics of Ligation. Whereas the results of both the T-state effect and ortho effects on equilibria of ligation are clear the kinetic effects are not so straightforward. In the binding of either 1-MeIm or carbon monoxide to 1-MeImHm there is virtually no difference between TMPFe and TPPFe as seen in Table III where $(k^{CO}_{1\text{-MeIm}})_{TMH} \cong (k^{CO}_{1\text{-MeIm}})_{TPH}$, etc. In the T-state system (B = DMI) the increased affinity of TMPFe for CO seems to depend upon the faster association rate (8.4×10^5 M^{-1} s^{-1} for TMPFe) compared to 8.2×10^4 M^{-1} s^{-1} for TPPFe. On the other hand, the very large difference in k^{DMI}_{DMI} for TMPFe vs TPPFe (a factor of 330) arises entirely from differences in dissociation rates. This is clearly an indication of stabilization of the planar heme state. This dissociation leads to a highly domed 5-coordinated structure. The rate is about 6000 times faster for TPPFe than for TMPFe. This very large dissociation rate increase confirms the postulate that the ortho effect operates at the bond breaking and bond making steps. It strongly suggests that, like the T-state effect, the ortho effect has to do with the stability of the planar heme. Therefore, any six-coordinated heme which is destabilized by some hindrance to planarity will show an increased dissociation rate because this strain is relieved. If this strain can be relieved in some other way, e.g. the ortho effect, then the dissociation rate will be smaller.

Although the picket fence hemes and tetramesityl heme have been considered similar in CO binding, based upon the behavior of the T-state systems there are rather large differences. The kinetics for the reactions of CO with PFPFe(DMI) and TMPFe-(DMI) are very similar in both on and off rates. But the corresponding MI complexes are very different. A chelated picket fence heme, $FePiv_3CIm$ reacts more than ten times faster than does TMPFe(MI) (or chelated TPPFe) although the dissociation rates are rather similar. The reasons for these higher rates remain a mystery. It cannot be a result of solvation because the solvation should be the same in the T and R state models and the discrepancy exists only in the R-state model compounds. Further evidence against a solvent effect as the source of the ortho effect is the observation that the ortho effect operates in the bond-making or breaking step and the solvation of the flat heme should not affect that step. It is part of the diffusion process.

Conclusion

We have shown that the T-state effect which accrues upon changing the proximal base from 1-methylimidazole to 1,2-dimethylimidazole and the ortho effect resulting from putting ortho substituents on the phenyl groups of tetraphenylhemes are tightly linked whereas the T-state and distal steric hindrance effects are not coupled. We attribute the ortho and T-state effects to constraints on planarity of the heme and the distal steric effect to hindrance of ligand diffusion to the active site position. Thus "T-state" and "ortho" effects operate in the bond making (or breaking) step whereas the distal steric effect operates on the diffusion steps.

When comparing model compounds to proteins, it is necessary to take these three effects into account. The ortho effect does not apply to proteins and thus a quantitative comparison of ortho substituted TPPFe complexes to proteins requires the inclusion of this special effect.

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